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Title: Protein Determination by Combustion		
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Contents

A. INTRODUCTION

1. Summary of Procedure

   Total protein is determined using nitrogen analysis. The sample is combusted with oxygen and the gases containing nitrogen oxides are collected in a ballast tank until a specified pressure is reached. Helium is used as a carrier and an aliquot of combustion gas containing nitrogen oxides is reduced to nitrogen. It is then passed through a tube containing magnesium perchlorate and sodium hydroxide on a silicate carrier to remove water and carbon dioxide. The nitrogen is measured with a thermal conductivity detector using helium as a reference. Nitrogen is then converted to protein using a conversion factor.

2. Applicability

   This method is suitable for the determination of protein content in fresh and processed meat and poultry products at levels ≥ 0.3 %.

   Note: Refer to 9CFR 439.1 for regulations involving food chemistry.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus
   a. Robot Coupé food processor - Robot Coupé U.S.A. Inc.
   b. Analytical balance - capable of weighing to 0.1 mg.
   c. Forced draft oven - Adjustable to 101 ± 1 °C.
   d. Three two-stage compressed gas regulators
   e. Ceramic combustion boats - Cat. No. 529-203, LECO.
   f. Foil Boat liners for liquid samples - Cat. No. 502-343, LECO.

2. Instrumentation
   a. LECO TruMac N with TruMac operating software

      Nitrogen conversion factor: 6.25 for meat and meat products.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted. The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents
   a. N-Catalyst - Cat. No. 502-049, LECO.
   b. Anhydrone (Magnesium Perchlorate) - Cat. No. 501-171. LECO.
   c. Lecosorb (Sodium Hydroxide on silicate carrier) - Cat. No. 502-174, LECO.
   d. Silicone grease - Cat. No. 501-241, LECO.
   e. Leak detection solution - Cat. No. 502-213, LECO.
   f. Copper Sticks - Cat. No. 502-304-500, LECO.
   g. Copper Turnings - Cat. No. 501-621, LECO.
   h. Glass wool for furnace filter packing - Cat. No. 501-081, LECO.
   i. Steel wool - Cat. No. 502-310, LECO.
   j. Cylinder - Compressed air, medical quality.
   k. Cylinder - Oxygen, 99.99% purity
   l. Cylinder - Helium, 99.99% purity

D. STANDARDS

Note: Equivalent standards / solutions may be substituted. Purity and counter ions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method. The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension data.

1. Standard Information
   a. EDTA -- LECO Cat. No. 502-092

      Use the conversion factor 6.25 to convert Nitrogen to protein.

E. SAMPLE PREPARATION

Process the sample until a homogeneous mixture is obtained. Take care not to lose moisture during processing.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples
   a. Weigh 1.0 ± 0.2 g of sample and/or QC tissue into a ceramic boat.
   b. Dry samples in a 101 ± 1 °C convection oven for 45 ± 5 min. After drying, load

samples into the instrument.

Note: If needed, samples may be cooled and/or stored in a desiccator until analyzed.

2. Procedure

   a. Analyze at least five blanks with boats, until three consecutive blanks have a stable value with a standard deviation of less than 0.002%. Blank correct using the last three consecutive values.

   b. Run 4 or more EDTA standards until three consecutive values have a Relative Standard Deviation of 0.2% or less. Use the last three consecutive values to drift correct.

   c. Load the set of samples into the protein analyzer.

   d. Analyze samples on instrument.

3. Instrumental Settings

   Note: The instrument parameters may be optimized to ensure system suitability.

   Prepare instrument by following the procedure outlined in the operator's instruction manual (i.e. pack reagent tubes, perform leak checks, etc.).

Furnace temperature:	1100 °C.
Lance flow:	≈1.8 L/min.
Purge flow:	≈4.2 L/min.
TE Cooler Temperature	5 °C.
Dehydration Time	0 seconds
Purge Cycles	2
Element Parameters	Nitrogen
Baseline Delay time	6 seconds
Minimum Analysis Time	35 seconds
TC Baseline Time	10 seconds

Burn Profile

Burn Cycle	Lance Flow	Purge Flow	Time (seconds)
1	Off	On	5
2	On	On	35
3	On	Off	End

Ballast:	
Equilibrate Time	30 seconds
Not Filled Timeout	300 seconds
Aliquot Loop	
Equilibrate Pressure Time	4 seconds
High Precision	Yes
High Speed	No

4. Sample Set
   a. EDTA
   b. Meat recovery
   c. Samples (up to 20)
   d. Safeguard (Meat recovery)

   Note: Additional QC can be run through the set to monitor reagents and instrument drift.

5. Calibration
   a. A standard curve must be established for each instrument method, and with each new lot of EDTA. The drift of the curve can be corrected as often as needed by analyzing three or more EDTA standards, and using the drift correction menu in the software.
   b. If the drift is off by 10% or more, re-run the EDTA calibration curve.
   c. The calibration is established using a 1/certified weighting and the simplest curve that minimizes the RMS error.

G. CALCULATIONS

Calculations are done using the instrument control software. The results are reported as % protein using the nitrogen factor of 6.25.

% Nitrogen x 6.25 = % Protein.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses, heat-resistant gloves, plastic gloves, laboratory coat.

2. Hazards

Procedure Step	Hazard	Recommended Safe Procedures
Unit operates at 220 volts AC and has a high voltage power supply	Can cause severe burns/electric shock	Turn instrument off and remove metal objects from hands and arms before reaching into the instrument cabinet.
Crucible combustion tube and reduction tube	Extremely hot (700 - 1150 °C)	Allow to cool or use suitable tool when they are hot
Pure Oxygen	Explosive greatly accelerates combustion	Remove all ignition sources from the laboratory area
Compressed gas cylinder	Explosive	Mount cylinders firmly and have two stage regulators attached before cylinder valves are opened.
Magnesium Perchlorate	Strong oxidizer, contact with flammable materials may cause ignition. Causes irritation to skin, eyes, and respiratory tract	Use in a fume hood.
Sodium Hydroxide	Causes burns to all body tissue. Corrosive. Reacts with some metals to form H_2	Use in a fume hood.

3. Disposal Procedures

Follow federal, state, and local regulations

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   a. Three consecutive values of EDTA run before the samples shall have a Relative Standard Deviation of 0.2% or less.

   b. The three consecutive blanks used for blank correction shall have a standard deviation of less than 0.002%.

   c. The meat recovery run before the samples shall have a ≤ 0.44% protein difference from the reference value.

d. The meat recovery run after the samples shall have a ≤ 0.33% protein difference from the last meat recovery run in the set.

2. Critical Control Points and Specifications

Record	Acceptable Control
Sample Condition	Sample must be dried before loading in the autoloader sample rack.
Forced Draft Oven	101 ± 1 °C.
Sample weight	1.0 ± 0.2 g. Note: Adjust sample weight if % total protein is out of calibration range.

3. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week per analyst when samples are analyzed.

ii. Records are maintained.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Investigate following established procedures.

ii. Take corrective action as warranted.

4. Sample Condition upon Receipt:

Unspoiled and sealed from the air.

J. APPENDIX

Minimum Level of Applicability (MLA): 0.3 %.

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Staff.